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CHROMOSOME STUDIES IN KUHNIINAE (EUPATORIEAE). I. BRICKELLIA

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(*continued from p. 267*)

Also, from the table it can be seen that those included give a good representation geographically, as well as sectionally, if we except the two from Brazil. Of those studied, one species is so far known only from Costa Rica and a second came from Honduras, Guatemala and Chiapas in the very south of Mexico. Sixteen came from the plateaus of central to south Mexico, and four from the deserts and mountains of Baja California. At least three are distinctly north Mexican while fourteen are largely from southwestern United States, though there is some overlap to the northern part of Mexico, while others extend northward. Two of the latter, as well as two other species, are definitely from the northern limit of the range of the genus.

Chromosome Number and Geographical Distribution

The number of chromosomes in 80 accessions, representing 41 species, is 9 at meiosis or 18 in the somatic cells. Not a single instance of polyploidy or aneuploidy was found in the examination of almost half of the species of the genus although some meiotic irregularities were observed.

This uniformity of chromosome number means the same number for a small shrub of the temperate zone like *B. microphylla* (Nutt.) Gray, which came from Asotin County, Washington, and thus approaches the Canadian border, and *B. argyrolepis*, endemic in Costa Rica. Though the latter country is deep in the tropical zone, this singular species of *Brickellia* was collected at an elevation of 1450 meters. Similarly all the perennial

species from Central America and south and central Mexico came from the plateau states, at elevations of from about six to eight thousand feet. Thus this genus of the western Cordillera has remained mostly montane and lives under temperate conditions. The southern species (omitting the two questionable species in Brazil), though they thrive in a more equable climate, do not actually endure truly tropical conditions. Somewhat exceptional to this pattern is the annual species, *B. diffusa*, which according to herbarium records, occurs all through the Caribbean. It has evidently become the weed of the genus and appears to be increasing its range. It can tolerate an altitude of less than 100 meters in tropical latitudes, although it does occur with other species on the higher plateaus in Mexico.

It is of interest here to note that Miranda (1944), exploring the eastern slopes of the Sierra Madre, found trees of *Nyssa sylvatica* in the northern part of the state of Puebla at altitudes of 1340 and 1650 meters. The geographical range of this species had been known previously to be from central Maine to Florida and westward to Ontario, Michigan, Arkansas, and Texas. Subsequently Sharp (1951), when comparing the Eocene Wilcox flora with some modern floras, stated that while 93 of 137 designated Wilcox genera were still extant in eastern United States, 48 were present in the flora of the eastern escarpments in Mexico. These figures show a high correlation between the vegetation of these plateaus of Mexico and that of the Carolinian Zone. In contrast to *Nyssa*, where the same species is found in northern United States and central Mexico, in the genus *Brickellia*, few species, if any, are common to two such different latitudes. In the introduction to his paper, Robinson (*l. c.*) states that only four species can be said to have broad ranges. These are the annual *B. diffusa*, and three perennials, *B. californica*, *B. grandiflora*, and *B. oblongifolia*. None of them can equal the range of *Nyssa*. In this there is perhaps seen the difference between a large plastic genus, such as *Brickellia*, with eighty to ninety species, and a more conservative one, as *Nyssa*, with six species in America, even when allowance is made for varying bases of specific determination. Or is it perhaps indicative of one genus having evolved with its species becoming widespread in the time of the more uniform mild climate prior to the Pliocene, while of

the other, great speciation at least has taken place in conjunction with and following the climatic deterioration of that time. The earliest fossil records of the *Compositae* are in the Pliocene while those of *Nyssa* are known from the Cretaceous.

Chromosome Number and Amount of Woodiness

Uniformity of chromosome number means the same number for small and large shrubs, perennials, from the herbaceous to those at least more woody at the base, and one annual herb. Study of wood anatomy of the genus has not been undertaken. The genus is believed to have no real tree-form, however, Robinson, as is often necessary for the monographer with only the herbarium specimens and collectors' notes, omitted the height of some species and gave three meters as the tallest. At least five species approximate this height, of which three shrubs, *B. argyrolepis*, *B. pacayensis* (Coult.) Robinson and *B. pendula* (Schrad.) Gray and one herb *B. nutanticeps* Blake, are included in the present study. As seeds of the first of these were sent to me by the collector[7] in Costa Rica, it was possible to obtain the information from him directly that "The seeds of *Brickellia* sent came from shrubs that were 8 to 12 feet high; most have stems that were about 2 inches in diameter at the base, but the lateral stems were from ½ to ¾ inch wide." Two seedlings of these were grown in the greenhouses of the Biological Laboratories of Harvard where they were necessarily limited in height by the glass roof. In two years they had produced stems that were one inch in diameter at the base. They were woody except for a very small pithy center. This fraction of pith however increased gradually in higher internodes. Professor E. Matuda wrote that *B. pacayensis*, which he collected at the margin of bush, among secondary growth, was an herbaceous shrub 2 meters high. *B. pendula*, as seen in several locations by the author, did not exceed that height, while stems of the more herbaceous *B. nutanticeps* reached 3 meters. Thus, it seems probable that the species, with an altogether limited range in Costa Rica, could be called (lacking definitive knowledge of the doubtful ones in Brazil) not only the most southerly species but the tallest and most woody. So far as is known at present, any species occurring to the south of it

[7] Mr. Jorge Léon.

in Panama, or in the West Indies, is found also north of Costa Rica, in Guatemala or Mexico. The unvarying number of chromosomes in these species would indicate that for this genus there is no correlation of chromosome number with the amount of woodiness. Confirmation of this is added in finding the same number in the one annual herb which it was possible to study, *B. diffusa.*

The herbaceous perennials vary from the more delicate and slender ones such as *B. brachyphylla* Gray or *B. grandiflora* (Hook.) Nutt. to those with stiff stems, six and nine feet in height, as *B. scoparia* (DC) Gray and *B. nutanticeps*, respectively. In this they resemble large-leaved species of *Eupatorium* of the temperate region. Others become more branched, form bushy clumps and have been referred to as woody at the base. In this genus there is no sharp line between this type of perennial and the shrub. Varying somewhat from these tall intermediates between the herbaceous and shrubby types, are the species of the subsection *Reticulatae*, four of which were collected. All have a thick woody caudex, giving rise to a number of stems with somewhat lanceolate leaves. In these characters they resemble *Liatris* of the *Punctatae* series. Also at least two herbaceous species, *B. monocephala* and *B. hymenochlaena* Gray, are known to have fleshy rhizomatous roots. Both of these have large heads and broad phyllaries, characters also occurring in the *Scariosae* series of *Liatris.*

Chromosome Morphology or Karyology

GENERAL OBSERVATIONS

The uniformity of chromosome number in the genus *Brickellia* does not necessarily mean a similar uniformity of karyotypes. Somatic divisions have been found in seventy-one accessions representing thirty-nine species, exclusive of varieties. In two of these species, mitosis was seen only in cells of young stylar tissue of the flower material fixed in the field. Of the other thirty-seven, studies have been made in comparable preparations of root-tips from germinating seeds or from seedlings as they were grown in the greenhouse.

Though striking contrast in the morphology of the chromosomes was not found, differences in their lengths do appear. To

the general classes, long, medium and short, applicable to the majority of the species, a fourth class, the short short has been added for a small minority. As adopted from the language of fiction writers, this compound term applies to the unusually small forms found in the short group. Again by combining observations of the position of the centromere with length, an attempt was made to classify* in table II the chromosomes of all species, of which sufficient material was available. In two from Baja California, *B. macromera*, *B. megaphylla*, and *B. oblongifolia* Nutt., var. *linifolia* (D. C. Eaton) Robinson, too few seeds proved viable to give an opportunity for adequate comparisons.

It is not difficult to differentiate chromosomes which are long and short but as transitions of those of either class to the medium are not abrupt, those on the border line are not easily determined. Also while early stages of long and medium chromosomes regularly had centromeres in median, submedian, and subterminal positions, the few short chromosomes of most species often appeared as straight rods. However an examination of enough cells usually showed them sometimes as V-shaped, indicating that they were mostly medianly constricted. In karyotypes having a majority of short chromosomes, further classification of these was possible in the well-studied species, *B. californica* (Torr. & Gray) Gray, represented by thirteen accessions. The longest pair of chromosomes in most species of *Brickellia* was recognizably long submedian (Lsm) and the next long median (Lm). In some the third in length were called long subterminal (Lst) while in other species such a pair debatably might be called medium in length (Mst). Similarly from the closely graduated sizes there were other questionable classes in listing the species. No claim for absolute differentiation of these merging forms is made. It is hoped that the overall pattern of the karyotypes can be compared in the photomicrographic figures, with the aid of the classification in table II. The numbers of the particular accessions studied, occurring in the

*The abbreviations adopted here are as follows: Using capital letters to express length, long chromosomes with median, submedian, and subterminal constrictions are represented as Lm, Lsm, and Lst respectively; chromosomes of medium length similarly constricted, as Mm, Msm, and Mst, and short chromosomes similarly, Sm, Ssm, and Sst. The new short short class becomes SS; when medianly constricted, SSm, and when terminally, SSt.

TABLE II.—Karyotypes in

Section	Subsection	Species	No. of the Accessions Studied	Pairs of Lm	Lsm	Lst	L with sat.	Mm
I		*B. diffusa*	III					1
VII	*Microphyllae*	*B. Nevinii*	I	1	1	1		2
		B. microphylla	Ia, II	1	1	1		2
		B. scabra	Ib	1	1	1		2
	Parvulae	*B. dentata*	I	1	1	1		2
		B. brachyphylla	II, III	1	1	1		2
	Reticulatae	*B. venosa*	IV	1	1	1		2
		B. oliganthes	I	1	1	1		2
		B. verbenacea	Ia	1	1	1		2
	Amplexicaules	*B. cuspidata*	I	1	1	1		2
		B. betonicaefolia	I	1	1	1		1
		B. amplexicaulis	I	1	1	1		1
	Brachiatae	*B. Coulteri*	IV		1	1	1	2
	Baccharideae	*B. laciniata*	I, II					1
		B. desertorum	I					1
		B. californica	I–XIII					1
		B. veronicaefolia var. *umbratilis*	I					1
		B. Palmeri var. *amphothrix*	I, II	1	1	1		2
	Coleosanthus	*B. Rusbyi*	II, III, IV					1
		B. glomerata	IV	1	1	1		2
		B. paniculata	I, III, X	1	1	1		2
		B. secundiflora var. *secundiflora*	II	1	1	1		2
		B. tomentella	IVa, VII	1	1	1		2
		B. argyrolepis	Ia	1	1	1		2
		B. adenocarpa var. *glandulipes*	III	1	1	1		2
		B. pacayensis	I	1	1	1		2
		B. floribunda	I					1
IX		*B. Greenei*	I					1
		B. Wislizeni	I	1	1	1		2
		B. peninsularis	I	1	1	1		2
		B. grandiflora	II, III	1	1	1		3
		B. incana	I, II					1
		B. lanata	I, II, III	1	1	1		2
X		*B. monocephala*	I	1	2			4

Species of *Brickellia*

Chromosomes								General Remarks
Msm	Mst	M with sat.	Sm	Ssm	Sst	SSm	SSt	
1	1		4			2		1 pr Sm may be Ssm
1	1		2					
1	1		2					
1	1		2					
1	1		2					Chromosomes all seem shorter than *B. brachyphylla*
1	1		2					
1	1		2					or are there 2 Lsm 0 Lst?
1	1		2					
1	1		2					
3	1		1					possibly 2 Msm and 2 Mm
3	1		1					possibly 2 Msm and 2 Mm
1	1		2					or 3 prs. S and 1 pr. M
1			4			2	1	1 pr. intermediate M to S? 2 or 3 prs. SSm?
1			4			2	1	1 pr. intermediate M to S? 2 or 3 prs. SSm?
1			2	1	1	2	1	1 pr. = Mst or Sst? 1 pr. Ssm or SSsm?
1			3	1		3		possibly a little longer than in *B. californica*
1	1		2					
1			3	1		3		1 pr. Ssm may be Sm in accession II
1	1		2					
1	1		2					
1	1		2					
2	1		1					or 2 Lsm and 1 Msm
2	1		1					or 2 Lsm and 1 Msm
2	1		1					
1			3	1		3		2 prs. M more nearly approach L than in *B. californica*
1		1	1	1	1	2	1	
2	1		1					1 pr. Msm = Lsm? and 1 pr. Mm = 1 Sm?
2	1		1					1 pr. Msm = Lsm? and 1 pr. Mm = 1 Sm?
2	1							
1		1	1	1	1	2	1	
1	1		2					
1	1							short arm of 1 pr. Lsm shorter than that of other

second column, refer to those given in table I along with their places of origin.

Due to immaturity or at times paucity of seeds received, equally abundant and favorable material was not obtained of each accession. Of some it was possible to repeat the studies and vary the technique more because for a time at least, plants grew successfully in the greenhouse. For contrast, preparations were made of *B. microphylla* by use of Meyer's (1945) paradichlorobenzene technique, and contraction of the chromosomes was obtained. This sometimes emphasized the centromere region in longer chromosomes to good advantage. However, reduction in length was detrimental to the study of the shortest ones in almost obliterating the constriction region as well as the delicate satellites. Such a limitation of its use in this genus can be seen in the clear figure of *B. microphylla* (fig. 35) from a particularly favorable aceto-carmine smear in which there were dozens of cells like the one photographed.

Though careful drawings were made of the karyotypes of fifteen species for comparison with a number of species of other closely related genera of the *Kuhniinae* (MS in preparation), only the photographic illustrations were used here. These were made of preparations without pretreatment for contraction and of fairly comparable metaphase stages. As this phase is of some duration, the chromosomes on the equatorial plate in any species may vary slightly in the amount of condensation. However, this margin of variation was taken into consideration by study of a sufficient number of cells and can be illustrated here by three cells of *B. glomerata* from one and the same Feulgen slide preparation (figs. 14, 42, 43). The first figure is characteristic of the karyotype of this species. The second shows slightly more condensation. The third, however, is most exceptional and equals or exceeds the contraction produced by paradichlorobenzene (cf. fig. 35). This cell, without the study of many others, would suggest quite erroneously, that *B. glomerata* was similar to one of a minority of species with very small chromosomes. This was the only occurrence of such contracted units resulting from Feulgen's, but following any technique, variations may occur which are recognized by ample study. While necessarily various stages were studied for the purposes of the interpretation of a

karyotype, for the listing in the table, comparable stages in each species had to be recognized. The best spread of chromosomes was selected for photography. A clear example of karyotypes of slightly more and less condensation is illustrated by the figures of similar dermatogen cells in the closely related species *B. amplexicaulis* Robinson and *B. betonicaefolia* Gray (see figs. 24, 25). The table compares species of one subsection with those of another subsection or section as well as different accessions of individual species.

ANALYSES OF KARYOTYPES TREATED BY SECTIONS

SECTION I. LEPTANTHODIUM.

From the few viable seeds of an herbarium sheet of *B. diffusa*, collected in 1946, enough mitoses were seen to allow a comparison of the chromosome types with other species, although none were very good for photography. Figure 1 gives the impression of consisting of units of almost equal size, not merely because they are a little crowded, but actually because no long chromosomes are included. At an earlier stage, before they had become well organized on the plate and were less condensed, three classes were recognizable by differences in length. Two medium chromosomes which were submedianly constricted were the longest of the set. In figure 31, they can be seen on the periphery at right and left bottom. In contrast, one of the same class, medianly constricted, appears at upper right and of the short class at upper left. Though no preparations of this species were attempted with paradichlorobenzene, one anaphase plate presented almost as clearly in miniature the forms of these short chromosomes (fig. 32). In all there are six of the medium class which appear paler somewhat diagonally across the bottom in the figure and could be resolved as a pair each with median, submedian, and subterminal constrictions, respectively. Continuing almost clockwise, there are six short ones with four of the short short class in the center.

This karyotype with smaller chromosomes in one annual species of the first section differs greatly from those in most species of perennials studied. However, it is very similar to a small minority of them.

SECTION VII. BULBOSTYLIS.

With this section begins the analysis of numerous perennial species. Of subsection *Clavigera*, only young flowering material of *B. scoparia* was collected in Mexico. Those meiotic chromosomes (fig. 50) are a little smaller than others collected at the same time and prepared in the same way (cf. figs. 51, 53). This suggests that the karyotype of *B. scoparia* could be expected also to be composed of somewhat smaller units.

SUBSECTION MICROPHYLLAE. In his introduction, Robinson referred to this subsection as containing four very closely related species which he recognized because they varied in types of pubescence and in their geographic ranges. Three of the four species were studied cytologically.

In figure 35 of *B. microphylla Ia* after treatment for contraction, the abbreviated types of the chromosomes can be recognized. Across the top of this plate are seen the two longest submedian and one of the long subterminal chromosomes with the mate of the latter lying just below it. The two long median chromosomes are at the lower right corner with two medium submedian ones occupying the right side. The four short chromosomes form a line diagonally from that corner to the upper

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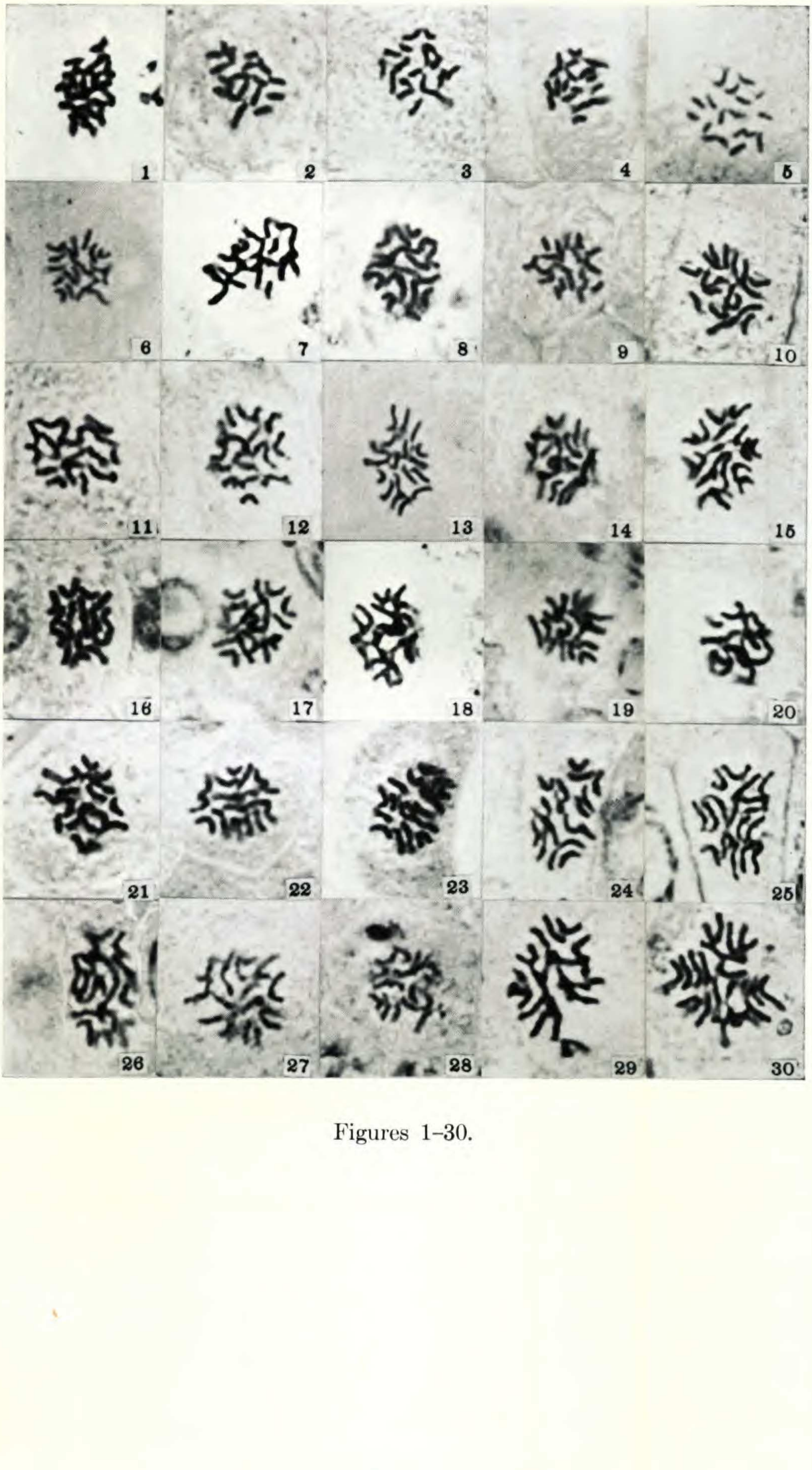
PLATE 1194

EXPLANATION OF FIGURES

Figs. 1–30 are from nearly comparable metaphase plates in root-tips. 1. *B. diffusa* III. 2. *B. Rusbyi* III. 3. *B. floribunda* I. 4. *B. laciniata* II. 5. *B. californica* XV. 6. *B. veronicaefolia* var. *umbratilis* I. 7. *B. dentata* I. 8. *B. brachyphylla* II. 9. *B. incana* I. 10. *B. microphylla* II. 11. *B. scabra* 1b. 12. *B. Coulteri* IV. 13. *B. Palmeri* var. *amphothrix* I. 14. *B. glomerata* IV. 15. *B. venosa* IV. 16. *B. oliganthes* I. 17. *B. verbenacea* Ia. 18. *B. cuspidata* I. 19. *B. lanata* I. 20. *B. secundiflora* var. *secundiflora.* II 21. *B. tomentella* VII. 22. *B. paniculata* I. 23. *B. peninsularis* I. 24. *B. amplexicaulis* I. 25. *B. betonicaefolia* I. 26. *B. Wislizeni* I. 27. *B. adenocarpa* var. *glandulipes* III. 28. *B. argyrolepis* Ia. 29. *B. grandiflora* III. 30. *B. monocephala* I. In this listing, where a species has the letter as well as a numeral, the preparation was from seed sent as a second collection, and where the letter b is appended, the seed represents the third attempt. Preparations were made as follows: Root-tips of seeds fixed in Karpechenko's, and stained in N. G. V., Figs. 7, 8, 9, 10, 12, 18, 23, 24 and stained in Feulgen's Fig. 30. Root-tips of seeds after Belling's, and stained in N. G. V., Figs. 1, 11, 20, 21, and stained in Feulgen's, Figs. 19, 28. All the rest of the root-tips were taken from plants, fixed in Belling's and stained *in toto* in Feulgen's. Figs. 2, 4, 13, 14, 26 at first time, Figs. 5, 15, 16, 22, 25, 27, 29 at a second time, and Figs. 3, 16, 17 at a third time.

The photomicrographs were taken with the use of a Zeiss microscope and a Homal IV lens, except figs. 4, 5, 6 and 13, for which the 90 × objective and 10 × ocular had been used, all figures 2300 ×, reproduced at 1650 ×.

It is a pleasure to acknowledge the assistance of Mr. Paul Brown in the photomicrography.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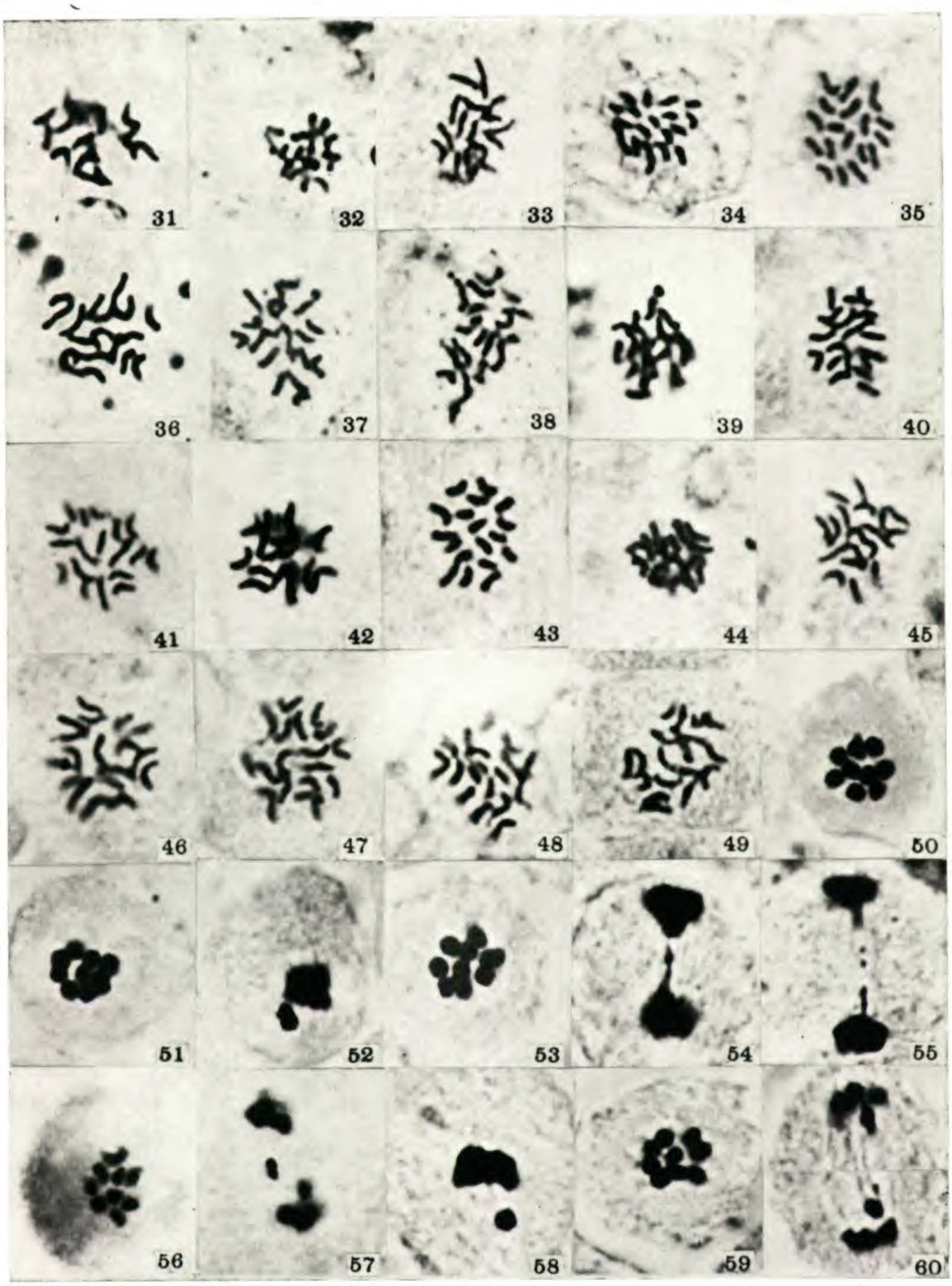
Figures 1–30.



Figures 31–60.

left, and the remainder are of medium length. It is impossible to distinguish any with subterminal centromeres among these. However, the preparations without pretreatment, especially the early metaphase stages, give confirmation of a medium pair subterminally constricted. In figure 10 of a cell of untreated *B. microphylla II*, one chromosome of this pair is clearly seen at the upper left corner. Indeed it is just this pair which was lying over the remnant of a nucleolus that persisted in a cell and so is believed to be the pair with nucleolar attachment. Comparing these cells after normal treatment with those of *B. scabra* (Gray) Nels. (fig. 11), no distinguishable differences can be recognized. In cells having the chromosomes more curved, they appear a little shorter and the plates are less crowded but very similar in each. In both these species, verified in two kinds of preparations, their karyotypes consist of gradually increasing units of length from short to long without striking breaks between any two classes. In preparations of *B. Nevinii I* (Fig. 33), which has just recently been received, there is no variation in the karyotype.

SUBSECTION PARVULAE. This subsection is represented by one accession of *B. dentata* (DC) Sch. Bp. from Texas and two of *B. brachyphylla* Gray from Colorado and Oklahoma respectively. Because *B. dentata* was received from one station only, prepara-

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PLATE 1195

EXPLANATION OF FIGURES

Figs. 31–49. All figures except 32 (anaphase) are metaphase stages from root-tips.

31, 32. *B. diffusa* III. 33. *B. Nevinii* I. 34. *B. desertorum* I. 35. *B. microphylla* Ia. 36. *B. pacayensis* I. 37, 38. *B. Coulteri* IV. 39. *B. californica* XVIII. 40. *B. californica* VI. 41. *B. Greenei* I. 42, 43. *B. glomerata* IV. 44. *B. argyrolepis* Ia. 45. *B. Greenei* I. 46. *B. Wislizeni* I. 47, 48. *B. incana* II. 49. *B. lanata* I.

Preparations were made as follows: Root-tips of seeds fixed in Karpechenko's, and stained in N. G. V., Figs. 36, 37, 38, 40, 47, 48. Root-tips of seeds after Belling's, and stained in N. G. V., Figs. 31, 32, 39, 44. Fig. 35 was stained in aceto-carmine following paradichlorobenzene treatment. All the rest of the root-tips were taken from plants, fixed in Belling's and stained *in toto* in Feulgen's, Fig. 46, at first time, Fig. 49, at second time and Figs. 42, 43, at third time, as referred to re Figs. 1–30. Figs. 33, 34, 41, and 45, at a later time.

Figs. 50–60. Stages of meiosis in pollen mother cells.

50. *B. scoparia* I. 51. *B. verbenacea* I. 52. *B. reticulata* I. 53. *B. glomerata* I. 54, 55. *B. glomerata* V. 56. *B. californica* I. 57, 58. *B. pendula* III. 59, 60. *B. adenocarpa* var. *glandulipes* Ia.

Figs. 50, 51, 53, 56, 59. Polar views of regular I metaphase plates. 52, 58. Lateral views of irregular I metaphase plates. 54, 55, 60. I telophase stages showing chromosome bridges. 57. I telophase with two lagging chromosomes dividing.

tions of root tips from three plants grown from seeds were studied. The chromosomes of the many plates seen (fig. 7), appeared shorter than in *B. brachyphylla* (fig. 8). When early stages of recently segmented spiremes, where the chromosomes are less condensed, were studied, approximately the same types were found as in that species. However, the metaphase of *B. dentata* did seem to represent a diminution in the overall chromosome length just as some species will be referred to as having fuller nuclei and generally longer chromosomes. In cells of *B. brachyphylla* it was clearly seen that the majority of chromosomes were isobrachial since as many as ten V-shaped chromosomes could be seen repeatedly in different cells. The shortest pairs (Sm) were very close to the chromosomes of medium length. Of the four heterobrachial pairs (one each of Lsm, Lst, Msm, and Mst), the long subterminal ones were seen particularly clearly in a Feulgen preparation, to thin out from the short arm into delicate achromatic strands, but no satellites were observed.

SUBSECTION RETICULATAE. Comparable preparations of two Mexican species, *B. oliganthes* (Less.) Gray and *B. verbenacea* (Greene) Robinson, and one from southwestern United States, *B. venosa* (Wooton & Standley) Robinson (figs. 16, 17, 15, respectively), were obtained from germinated seeds and roots of young plants. In no definite way could these karyotypes be distinguished from one another or from those of the *Microphyllae* described previously. In studying numbers of cells, one did not always obtain exactly the same analyses of constriction types but they were usually consistent as to the class lengths. In a third and rarely collected Mexican species, *B. reticulata* (DC) Gray, the seeds were immature and mitotic figures were seen only in the smaller cells of young flower tissue. It was hardly possible to analyze this karyotype. This was regrettable since irregularities in meiosis (fig. 52) found in this plant were possibly indicative of some hybridity. Further interest in it lies in the fact that Robinson considered this species, along with *B. venosa* and another from Guatemala, *B. Kellermani* Greenman, to be very close to *B. oliganthes* in technical characters. Elsewhere (Gaiser, 1952) the significance of my collections has been discussed.

SUBSECTION AMPLEXICAULES. *Brickellia cuspidata* Gray stands apart from the other three species in this sub-section by its cori-

aceous, bright green leaves and is rather distinct by having a marked cusp at the tip of the leaf. Unfortunately only a very few mature seeds were received from Guadalajara this past February. Cells in preparations from them were more satisfactory for study than for photography (fig. 18). The karyotype, however, fell in line with that predominating in the previously discussed groups, comparing more favorably with them than with others of this subsection.

Two of the three allied species, with membranaceous, obscurely pubescent leaves, *B. betonicaefolia* and *B. amplexicaulis*, have been separated in the key by the absence or presence of stipitate glands on the pedicels, respectively. This character is reviewed in a later section of the paper. Cells of *B. betonicaefolia* showed well filled nuclei and moderately large plates as is usual when the chromosomes are longer. Figure 25 of a well spread plate in a rather flattened cell of the dermatogen layer shows a greater predominance of long and medium chromosomes than in previously discussed species, and has only two short ones. These lie below the middle line in the center of the plate. Repeated classification in different cells left questionable the number of hetero- and iso-brachial chromosomes of medium length. Though most of the studies favored three Msm and one Mm, these two classes may be equal. Five instead of four medium chromosomes with the corresponding reduction in number of short ones account for the larger amount of chromatin. Of *B. amplexicaulis*, a very similar narrow cell in the dermatogen is included (fig. 24). Though the chromosomes are of a slightly more condensed stage, the karyotype is obviously very like that of *B. betonicaefolia* and again there are only two short chromosomes. These appear in the figure as two short rods slightly overlying others, one prominently at the center right and the other more faintly, directly opposite, at center left.

SUBSECTION BRACHIATAE. To the six species of this subsection in Robinson's treatment, two more have been added. *B. megaphylla* Jones (1933) is reported by Blake (1945) to have been seen and described by Robinson who considered it close to *B. hastata* Benth. *B. urolepis* Blake (1942) has also been described as being a close ally of that species. All have been reported in limited and widely separated localities in Mexico without over-

lapping (e. g., San Luis Potosi, Coahuila, Sinaloa and particular islands or bays of Baja California) except *B. Coulteri*, which occurs more widely from Arizona to Puebla in Mexico. As mentioned above, only a very limited number of seeds of *B. megaphylla* and *B. Coulteri* from Baja California were received. Of the former, insufficient mitoses were seen for an analysis of its karyotype to be safely made at this time. Of the latter, comparisons were possible in at least four root-tips. While not found in every division figure, preparations of *B. Coulteri IV* frequently showed one chromosome with a conspicuous satellite and sometimes the pair were evident. Both were visible in the cell photographed (fig. 12) though the one which is not on the periphery of the plate is less evident. It was more often possible to find cells which could be photographed, showing clearly one chromosome with a satellite (figs. 37, 38). That the chromosome is long and heterobrachial is shown in all three figures. Also from analyses of them and other cells, we believe it and its mate replace the pair of long median chromosomes. The small appendage is distal to a short arm and these two together are about equal to the long arm of the chromosome. No evidence was found of complete separation, as of a fragment in a cell. It would have been interesting to have had more than one accession for study, especially since *B. Coulteri IV* was one of a small minority of herbarium specimens lacking macroscopic evidence of sessile glands.

SUBSECTION BACCHARIDEAE. The first distinctly different karyotype of a perennial *Brickellia* was found in this subsection of seven species, five of which were studied.

Of *B. laciniata* Gray, it was possible to make comparisons of two accessions received at very different times in this investigation and from separated localities in Texas and Arizona. In the figure shown of the latest accession (fig. 4), one of the longest pair of chromosomes is at the upper right and the next longest at the center top. The respective mates of these two pairs are at lower right but not exactly in focus. These four chromosomes compare favorably with those designated medium (Msm and Mm, respectively) in previous species. Of the seven remaining pairs, it was difficult to determine whether one was intermediate to short and whether of the shortest (SS) there were two or three

pairs. Certainly two little straight rods, lacking any apparent median constriction, would qualify. Though these were determined as SSt they may really be SSm. The interpretations of the two collections is given in table II.

B. californica probably has the widest distribution of any species in the United States, with a range extending eastward from California to western Texas and Colorado and northward to Utah. It was the first species received and more viable material of it has come in than of any other species. Most of the specimens, which came from four different states, fell under var. *californica*. Two of the accessions, XIVc from Mesa Verde National Park, Colorado, and XVII from Coronado National Park, Arizona, tended towards var. *tenera*, but it was uncertain whether they merely represented forms growing in shade. It was possible to examine good mitotic figures of thirteen accessions. The many preparations gave adequate opportunity to make comparisons at different stages and certainly strengthened the conviction that a distinctively different karyotype existed in this subsection. In figure 5 of *B. californica XV*, the chromosomes are approximately as short as those of *B. laciniata*, and again there are none of the long class. The two across the top represent the longest pairs, one with submedian constriction to the left and median to the right. The other two members of these pairs are respectively at lower right and in the center, both not quite in focus for their entire lengths. These chromosomes are certainly not comparable to anything longer than the medium ones seen in many of the previously mentioned species and are, therefore, classed as Msm and Mm. The remaining fourteen appear short and it may be difficult to draw a line between two further classes in this figure. It was for this species, however, as seen in a number of the shortest chromosomes mostly distinguishable at the lower left, that the new class short short was established. A closer analysis was possible because of the many cells available in different and especially earlier stages. Although not a very early metaphase, the cell photographed (fig. 40) of *B. californica XIII* does show the chromosomes of this species when still more elongate. The classification was best made from very favorable preparations of accession XIVc.

From examinations of such figures it was realized with some surprise that the types of these short chromosomes were proba-

bly parallel to the medium ones of the more general karyotype in other species, but on a lower size scale. The short short would then be a reduction of that short class. If one compares these two figures of *B. californica* with the figure of *B. microphylla* after treatment by paradichlorobenzene, and the singular cell of *B. glomerata* in a Feulgen preparation, the likeness is certainly striking (cf. figs. 35, 43). With the suggestion of reduction of the chromatin material in this species, it was more pertinent to find occasionally in cells of at least two preparations, a fairly large terminal body on one of the chromosomes. Fig. 39 shows a cell of *B. californica XVIII* where it is conspicuous because of its position on the periphery of the plate. As it was not found repeatedly in other accessions, it is improbable that it is a regular feature of the karyotype of the species though it may be of this accession. The second collection, XVII, from a higher elevation in the park, which might qualify for variety *tenera*, did not show it. It is larger than the satellite of *B. Coulteri*. No evidence was seen of its becoming completely detached as a fragment, though that may occur. It is certainly suggestive of a means of lost particles which would result in a reduction in the length of the chromosomes.

A collection recently received from Riverside County, California, compares well with the description of *B. desertorum* (Coult.) Robinson and with other specimens so annotated in the Gray Herbarium. In preparations from germinated seeds of it, there was found to be a predominance of short chromosomes similar to that of the two previously described species. The six shortest units across the center of the plate (fig. 34) are considered to belong to the short short class and eight slightly longer ones, almost surrounding them, as short chromosomes. The longest pair, in the bottom row, are clearly Msm and the pair alternating with them, Mm, though they appear slightly fore-shortened. This karyotype could not be recognized as differing from that of *B. californica*. A second recent collection received from San Bernardino County, California, differs from the first in having heads containing a few more florets and larger leaves, with slightly less indumentum. In these characters it approaches *B. californica*. It would be necessary to have many more specimens to determine whether these two species inter-

grade in certain regions. Certainly the karyotype would be of no help in distinguishing the two entities.

The species *B. veronicaefolia* is apparently as abundant in Mexico as *B. californica* is in United States, with a range from Durango to Oaxaca. None of fifteen collections of varieties *veronicaefolia* and *senilis* Robinson, made by the author in Mexico, proved viable, as the seeds were not mature. Of one accession of variety *umbratilis* Robinson, obtained from north central Mexico, a few of the terminal heads contained filled achenes. Preparations from these, made at the same time and by the same techniques as a number of those of *B. californica*, show a rather similar karyotype. On the whole, the chromosomes appear a little longer in figure 6, but when compared with the earlier stages of that species (see fig. 40), there is not a great deal of difference. However, if the cell is compared with one of almost the same form and size of *B. betonicaefolia*, which has been referred to above as having longer chromosomes, the difference in chromatin content is distinctly noticeable.

The fourth species, *B. Palmeri* Gray, was represented by two collections of the pubescent variety *amphothrix* Robinson from Durango. Their chromosomes seem as identical as their taxonomic characters. However, the karyotype (fig. 13) differs from other species of this subsection. The almost radial arrangement in this cell again shows that long and medium chromosomes are more abundant than the short. Analyses of their types agreed better with many of those of the following subsection. This was not surprising from the taxonomic viewpoint after examining herbarium specimens and seeing seedling plants growing next to those of *B. glomerata* in the greenhouse. Thus we believe cytological evidence strongly supports a different disposition of this species as will be indicated later.

SUBSECTION COLEOSANTHUS. With heads of approximately the same size as those of the previous subsection, Robinson had separated this group of species largely on the basis of leaves. They are given as ovate, lanceolate or elliptical with the cauline ones petiolate, while those of the *Baccharideae* are ovate, rhomboid or suborbicular-reniform and more often small.

The first species, *B. Rusbyi* Gray, is found in New Mexico, southern Arizona, and a little beyond the boundary in northern

Mexico. This was studied in three accessions representing both New Mexico and Arizona and was found to have a complement of chromosomes with those of the shorter length predominating (fig. 2). It is difficult to see that this karyotype varies much from that of *B. californica* or *B. laciniata*, although there may be a more definite break between the four longest chromosomes and the rest. It was not easy to make a final decision in that small realm of difference between medium and short length.

The remaining twenty-three species of this subsection all belong to Mexico and Central America, except the last, *B. floribunda* Gray, which has the same geographic range as *B. Rusbyi*. Other similarities of these two species will be discussed in a later section. While *B. floribunda* was also received from both states, the specimen from New Mexico was immature and thus the karyotype has been seen only in preparations of Arizona material. In numerous cells seen, the open arrangement resulting from short unentangled chromosomes resembles that of *B. Rusbyi*. There were always four longer chromosomes which are not adequately shown in figure 3, because three of them curve out of the plane of focus. In one cell, two of these occurring endwise with one additional short one, occupied the full diameter of the plate. The six short short chromosomes were frequently V-shaped and were considered to have median constrictions. Of the other eight, one pair had possibly submedian and the other three median constrictions. On the whole it is doubtful whether the chromosome set of *B. floribunda* could be distinguished from either that of *B. Rusbyi* of this same subsection or *B. laciniata* and *B. californica* of the previous one. More accessions of it would help confirm or negate the apparent distinctiveness of the four longest members.

In strong contrast are four species from Mexico as well as *B. adenocarpa* from Guatemala and *B. argyrolepis* from Costa Rica. These all have a complement of longer chromosomes. Three figures of *B. glomerata* (figs. 14, 42, 43) have been referred to above as occurring in one and the same Feulgen preparation, the second one showing slightly more condensed chromosomes than the first. The third cell is singular in the unusual amount of contraction involved. It is about equal to that following the paradichlorobenzene treatment of *B. microphylla* (see fig. 35).